GEORGIA INSTITUTE OF TECHNOLOGY — OFFICE OF CONTRACT ADMINISTRATION

PROJECT ADMINISTRATION DATA SHEET

[X] ORIGINAL [ ] REVISION NO. _____

Project No. G-33-W05 R5983-5A0 GTRC/GHXX DATE 7 / 22 / 86

Project Director: Dr. Sheldon W. May School/XXX Chemistry

Sponsor: DHHS/PHS/NIH/NHLBI

Type Agreement: Grant No. 5R01 HL28167-05

Award Period: From 7/1/86 To 6/30/87 (Performance) 9/30/87 (Reports)

Sponsor Amount:	This Change	Total to Date
Estimated: $		$ 201,045
Funded: $		$ 201,045

Cost Sharing Amount: $ N/A Cost Sharing No: _____

Title: Novel Antihypertensives: Rational Design and Evaluation

ADMINISTRATIVE DATA OCA Contact E. Faith Gleason X-4820

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Defense Priority Rating: N/A Military Security Classification: N/A

(or) Company/Industrial Proprietary: _____

RESTRICTIONS

See Attached N/A Supplemental Information Sheet for Additional Requirements.

Travel: Foreign travel must have prior approval — Contact OCA in each case. Domestic travel requires sponsor approval where total will exceed greater of $500 or 125% of approved proposal budget category.

Equipment: Title vests with GIT

COMMENTS:

SPONSOR'S I. D. NO. 02.108.001.86.025

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SPONSORED PROJECT TERMINATION/CLOSEOUT SHEET

Date 8/5/87

Project No. G-33-W05 School/~~Lab~~ ~~X~~ Chemistry

Includes Subproject No.(s) N/A

Project Director(s) Dr. Sheldon W. May GTRC /~~XXX~~

Sponsor DHHS/PHS/NIH/NHLBI

Title Novel Antihypertensives: Rational Design and Evaluation

Effective Completion Date: 6/30/87 (Performance) 9/30/87 (Reports)

Grant/Contract Closeout Actions Remaining:

- [ ] None
- [X] Final Invoice or Final Fiscal Report
- [ ] Closing Documents
- [ ] Final Report of Inventions
- [ ] Govt. Property Inventory & Related Certificate
- [ ] Classified Material Certificate
- [ ] Other _____

Continues Project No. _____ Continued by Project No. G-33-W06

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Duane H.
Russ M.

FORM OCA 69.285

SECTION IV PROGRESS REPORT SUMMARY	GRANT NUMBER HL28167	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR Sheldon W. May	PERIOD COVERED BY THIS REPORT FROM 7/1/86	THROUGH 6/30/87
NAME OF ORGANIZATION Georgia Institute of Technology		

TITLE *(Repeat title shown in item 1 on first page)*
Novel Antihypertensives: Rational Design and Evaluation

(SEE INSTRUCTIONS)

Publications

"Ascorbate Depletion as a Consequence of Product Recycling During Dopamine-B-Monooxygenase Catalyzed Selenoxidation," S. W. May, H. H. Herman, S. R. Roberts, and M. J. Ciccarello, Biochemistry, 26, 1626 - 1633 (1987).

"Interaction of Dopamine-B-Monooxygenase with Substituted Imidazoles and Pyrazoles: Catalysis and Inhibition," S. R. Sirimanne, H. Herman, and S. W. May, Biochemical Journal, 242, 227 - 233 (1987).

"Mechanistic Studies on Dopamine-B-Monooxygenase Catalysis: N-Dealkylation and Mechanism-Based Inhibition by Benzylic Nitrogen-Containing Compounds. Evidence for a Single Electron Transfer Mechanism," K. W. Wimalasena and S. W. May, J. Amer. Chem Soc., in press (1987)

"Demonstration of the Potent Antihypertensive Activity of Phenyl-2-aminoethyl Sulfides," H. H. Herman, S. H. Pollock, L. C. Fowler and S. W. May, J. Cardiovasc. Research, submitted for publication.

"Mechanistic Studies on Dopamine-b-Monooxygenase Catalysis: N-Dealkylation and Mechanism-Based Inhibition. Evidence for a Single Electron Transfer Mechanism", Fed.Proc. 45, 1537 (1986)

"The Antihypertensive Activity of Selenium-Containing Analog of Phenylpropylamine", Fed Proc. 46, 1290 (1987)

"Mechanistic and Specificity Studies on Dopamine Beta Monooxygenase Catalysis", Fed Proc. 46, 0000 (1987)

"Antihypertensive Activity and Ascorbate Depletion via Product Recycling by DBM-Targeted Selenides", Fed Proc 46, 0000 (1987)

PROGRESS REPORT

The goals of our research program are: (1) to answer key questions regarding the biochemical mechanisms responsible for the antihypertensive activity we have demonstrated for certain compounds of our design; (2) to bioassay the potential antihypertensive activity of other compounds we have already designed and synthesized based on the ideas of this proposal; and (3) to extend our efforts by continuing to design and evaluate other classes of compounds potentially capable of exhibiting antihypertensive activity through the modification of adrenergic neuronal activity.

Substantial progress has been made in all of these areas. The following paragraphs summarize our progress during this second year of the current project period.

Experiments in Chromaffin Vesicle Ghosts. As anticipated, over the course of this past year we have greatly expanded this aspect of our research program. In our view, such experiments will serve to forge the critical link between the enzymology of our novel neurotransmitter analogs and the potent biological activity we are observing in our animal studies. A specific focus at this time are our selenium-containg compounds, with corresponding experiments being carried out with the sulfide cognates. The working hypothesis we are testing is as follows. We visualize that the biological effects we observe with our selenides, sulfides, olefins, etc. (e.g., antihypertensive activity in SHR) are initiated through a mechanism involving initial uptake into neuronal cells, followed by uptake into neurotransmitter storage vesicles. After uptake, we wish to demonstrate that by virtue of their ability to act either as DBM substrates or inhibitors, our compounds effectively reduce the amount of norepinephrine (NE) available for release and post-synaptic activation of adrenergic receptors. We expect that specific biochemical processes will be operative for each of the several classes of compounds we are developing. For example, in the specific case of the selenides, we anticipate that DBM-mediated turnover of the selenides results in ascorbate depletion within vesicles, the striking discovery we have confirmed in our in-vitro enzymological work, and now wish to confirm this in vesicle ghosts.

We have now conclusively demonstrated that our selenides and sulfides are indeed actively taken up across the vesicle membrane in a process which is strictly dependent on Mg-ATP. Importantly, we have developed the technology to simultaneously determine catecholamines and ascorbate levels using HPLC/electrochemical detection, and to separately determine the concentration of each of these species inside or outside of the vesicles. In a manuscript under submission to J. Biol. Chem., we demonstrate conclusively that the DBM in our vesicles is mainly (80%) the membrane-bound species; that our vesicles are fully functional in taking up DA and converting it to NE; that uptake is strictly Mg-ATP dependent; and that conversion is requires DBM turnover and electron consumption from ascorbate. Furthermore, we demonstrate that external ascorbate can support DBM turnover within the vesicles, with electrons flowing across the membrane serving to recycle internal ascorbate. A stoichiometric relationship exists between external electrons available and total NE production. Importantly, our data clearly indicate that it is the internal ascorbate which functions as the immediate reductant for facile DBM turnover; a much slower endogenous rate of NE production does occur in the absence of ascorbate, and this is attributable to slow catecholamine-supported DBM turnover.

It is clear from these reults that membrane-bound DBM within chromaffin vesicles indeed utilizes directly internal ascorbate as the immediate reductant supporting catalytic turnover. This is a critical point if our selenides are to deplete ascorbate in adrenergic neurons, where the DBM is membrane-bound and not the soluble enzyme universally isolated by biochemists from adrenal medulla. In the past two months, we have established that the selenides are actively transported across the vesicle membrane and that they do, indeed, cause a depletion of reduced ascorbate within the vesicles concommitant with DBM turnover. Depletions of about 80% have been observed in our experiments, and we are now carrying out comparison studies with our sulfide substrates. Furthermore, we have also shown that the sulfides are indeed converted to sulfoxides by DBM turnover within the vesicles, an important prerequisite if the sulfides are indeed functioning as "false transmitters" as we hypothesized in our original proposal.

Thus, we feel we are well on our way to obtaining answers for key questions regarding the biochemical mechanisms behind the activities of our compounds. We anticipate that the data we obtain with vesicle ghosts during the coming year will continue to be of great value in this regard. Furthermore, we have now set up for adrenal cell culture in our laboratory using a borrowed laminar flow hood, and after a number of practice protocols and some training from colleagues at Emory, we have successfully cultured adrenal cells in our laboratory. Thus, we can proceed actively with this aspect of our program during the comimg year.

Enzymological Studies. The complete description of the enzymology of our selenides, documenting their remarkable ability to deplete ascorbate via product recycling, was published last month in Biochemistry (see list of publications)

We have now completed the full mechanistic analysis of turnover and suicide inhibition with our novel class of N-dealkylation DBM substrates; a major paper on these has been accepted for publication in the Journal of the American Chemical Society and is now in press. To summarize briefly, we discovered a new class of DBM substrates containing benzylic nitrogen atoms on which DBM exhibits a heretofore unknown catalytic activity -- oxygenative N-dealkylation by the enzyme to produce aniline and an aminoaldehyde. We extensively studied the mechanism of this N-dealkylating activity and firmly established that these compounds are also potent mechanism-based enzyme inactivators. It is clear from our mechanistic work that the enzymatic reaction is initiated by a single electron oxidation of the substrate to generate a nitrogen cation radical. After this initial step, the activated substrate either loses an alpha hydrogen via base-catalyzed abstraction to generate a carbon-centered radical which is ultimately hydrolytically cleaved to produce the product pair of aniline and 2-aminoacetaldehyde, or by rearrangement of the radical cation, causes irreversible inactivation of the enzyme via covalent attachment to the active site. An aspect of the N- dealkylation activity for DBM which is quite striking to us is that this is a direct model for the peptidylglycine amidating monooxygenase (PAM) enzyme which is responsible for generating the C-terminal amide present in so many peptide hormones of neurological importance. Enzymology on PAM has just started to attract a high degree of interest in the literature, and it isstriking that PAM is a copper and ascorbate requiring monooxygenase quite analogous to DBM. During the coming year we plan to carry out further scoping of the relationship between our N-dealkylase activity of DBM and PAM, and the implications of this relationship to neurochemical processing and drug design.

In January, we published (in Biochemical Journal; see publication listing) our first report on the novel class of DBM substrates and inhibitors containing nitrogen heterocycles such as imidazole, imidazoline, and pyrazole in place of the amino moiety at the side chain terminus. This work has opened up a new direction leading to both novel substrates undergoing dealkylation, and novel potent competitive inhibitors. Thus, as we report, compounds such as p-hydroxy benzylimidazole (PHBI) are active substrates, undergoing oxygenolytic cleavage to 4-hydroxybenzaldehyde and imidazole. In contrast, when the terminal amino group is replaced with pyrazole, a functionality which is uncharged at the pH of the enzymatic reaction, the analog possesses potent DBM competitive inhibitory activity. Evidence in hand is suggestive that the pyrazole moiety is interacting with the copper at the active site of DBM. We are now extending these findings and are synthesizing 2'-substituted imidazole cognates as well as analogs with altered side chain lengths and bis-imidazole compounds. We have carried out testing in SHR with the parent imidazole and pyrazole compounds but did not observe marked

antihypertensive activity. We will thus be very interested in obtaining bioassay results on these other heterocyclic analogs.

A number of other things have been accomplished in the enzymological aspects of our program. For example, we have synthesized quite a number of analogs of our prototypic olefin inhibitor, PAME, and kinetic characterization of these is in progress. Non-aromatic DBM substrates have been prepared and we have accumulated kinetic data on these as both substrates and inhibitors. Deuteriun isotope effect measurements are being carried out with N-dealkylation substrates. Finally, we have now repeatedly used our new isolation method for DBM; using the purified chromaffin granules which we must prepare anyway for our uptake experiments, our short, fast preparative method employing FPLC techniques yield enzyme of 60-85 Units per mg, from much less starting material.

Bioassay of Antihypertensive Activity. Both chronic and acute dosing protocols are now being used on a continuuing basis, and we have become quite expert in the surgical techniques for cannulation and direct bp measurement. We have expanded our administration routes to now include i.p., i.v., subcutaneous, oral, and osmotic pump routes. Using direct measurements in cannulated SHR, we have in hand definitive, statistically significant antihypertensive activities for all four of our prototype sulfides (PAES and its HO, Me, and HOMe analogs), and a paper on this has been submitted to J. Cardiovas. Pharmacol. Striking activity in both acute and chronic dosing over a two week period has been obtained with our prototypic selenium compound, PAESe. In adjunctal experiments, whole hearts were removed at the end of the dosing period, and using HPLC with electrochemical detection, are able to demonstrate a marked change in sympathetic nerve-ending catecholamines in the drug-treated animals. We have found a >50% reduction in norepinephrine and a >70% reduction in epinephrine from hearts of animals treated with PAESe which correlates with the effects on blood pressure and heart rate already noted. In the case of PAME, similar effects have been observed. During the coming year we plan to continue these bioassays with our compounds using all of these protocols including implanted osmotic pumps to continuously deliver compound doses in SHR.

Finally, in a novel approach we did not anticipate in our original proposal, we have successfully carried out enantiomeric resolutions for both MePAES and MePAESe. Preparative scale resolutions have been completed and we have completed X-ray struture determinations of the absolute configurations of the enantiomers. This is highly exciting to us, since we anticipate markedly different uptake interactions for the enantiomers of these compounds, but essentially no enzymological differences. Thus, having available these enantiomers in quantity sufficient for bioassay presents a telling probe of the biochemical mechanisms operative for our compounds. We will begin testing the individual enantiomers of PAESe within the next two months, and will then proceed to the sulfides. In the future, this approach can be extended to the other classes of compounds (olefins, N-compounds) as well.